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TOWNSEND and TOWNSEND and CREW LLP

By: Lata Olivier

PATENT
Attorney Docket No.: 02307O-125630US
Client Ref. No.: SF 2002-071-2

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of:

HE et al.

Application No.: 10/678,639

Filed: October 3, 2003

For: METHODS FOR TREATING CANCER BY INHIBITING WNT SIGNALING

Customer No.: 20350

Confirmation No. 7591

Examiner: BRISTOL, Lynn Anne

Technology Center/Art Unit: 1643

DECLARATION UNDER CFR § 1.131

Mail Stop Amendment
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

We, Biao HE, Liang YOU, Zhidong XU, and David M. JABLONS, being duly warned that willful false statements and the like are punishable by fine or imprisonment or both, under 18 U.S.C. § 1001, and may jeopardize the validity of the patent application or any patent issuing thereon, hereby declare and state as follows:

1. All statements herein made of our personal knowledge are true and statements made on information or belief are believed to be true. **Exhibits 1-11**, attached hereto, are incorporated herein by reference.

2. We are fully aware of the contents of U.S. patent application Ser. No. 10/678,639 ("'639 Application") and its priority provisional application Nos. 60/491,350 ("'350 Application"), filed on July 31, 2003 and 60/509,037 ("'037 Application"), filed on October 4, 2002.

3. We are co-inventors of the invention disclosed in the '639, '350, and '037 Applications and of the invention claimed in the elected and pending claims 31, 32, 34, 36, and 37.

4. The claimed invention relates, *inter alia*, to a method of inhibiting the growth of a cancer cell that overexpresses a dishevelled-3 (Dvl-3) protein comprising contacting the cancer cell with an agent that inhibits Dvl-3 expression thereby inhibiting the growth of the cancer cell.

5. We have read and we are familiar with the Office Action mailed May 1, 2007. We understand that in the Office Action mailed May 1, 2007, the Examiner has rejected claims 31, 32, 34, and 37 under 35 U.S.C. § 102(e) as allegedly being anticipated by Alsobrook *et al.* (U.S. 2003/0229016, based on U.S. application Ser. No. 10/307,928 ("'928 Application"), filed December 2, 2002; "Alsobrook"). According to the Examiner, Alsobrook teaches methods for treating a cancer cell such as a lung cancer cell or a breast cancer cell using an siRNA which inhibits expression of a splice variant of a dishevelled-3-like protein.

6. At the time we first conceived of this invention, we were employed by the Assignee of the '639, '350, and '037 Applications, The Regents Of The University Of California. All the activities described in this declaration took place in the United States.

7. Prior to December 2, 2002, we discovered that Dvl-3 was overexpressed in cancer cells. We showed this by overexpression of the Dvl-3 mRNA and overexpression of Dvl-3 protein in cancer cells.

8. Attached to this Declaration is **Exhibit 1**. **Exhibit 1** includes a page of a laboratory notebook shared by Dr. You and his part-time technician, Keeling Zang, in which they recorded part of their work on the "dishevelled" project. This page of the notebook was written prior to December 2, 2002. The dates have been redacted. Page 1 of **Exhibit 1** shows the experimental details of performing a microarray gene expression (Atlas Cancer Array) and concludes with "good result. worked." Page 2 of **Exhibit 1**, entitled "Identification of Dvl-3-Overexpression Using Microarray Gene Expression (Atlas Cancer Array)" shows Dvl-3 overexpression indicated by spots marked "9." The result thereof identifies Dvl-3 mRNA as one of the genes overexpressed in a tumor tissue when compared to normal tissue. The result shown on page 2 in **Exhibit 1** was obtained prior to December 2, 2002.

9. Attached to this Declaration is **Exhibit 2**. **Exhibit 2** is a page of a laboratory notebook in which Dr. You and his part-time technician, Keeling Zang, recorded part of their work on the "dishevelled" project. This page of the notebook was written prior to December 2, 2002. The dates have been redacted.

10. Page 1 of **Exhibit 2** shows an experimental protocol for determining expression levels of a Dvl-3 proteins in lung tissue samples by Western blot analysis. Tissue samples "10890280 NT" and "0890280 TT" represent lung cells from normal tissue ("NT") and tumor tissue ("TT"), respectively. We used anti-Dvl-3 antibodies ("Primary Ab mouse DVL3 1:400") to detect the Dvl-3 protein in these lung cells. The Western blot result shown on page 2 of **Exhibit 2** demonstrated overexpression of Dvl-3 protein in lung tumor cells (lane marked "0280TT") compared to normal lung tissue (lane marked "0280 NT"). The result shown in **Exhibit 2** was obtained prior to December 2, 2002.

11. Page 1 of **Exhibit 3** shows an experimental protocol for determining expression levels of a Dvl-3 proteins in mesothelioma samples (identified by numbers "10899111," "10891021," "10799111," and "10899120") by Western blot analysis. Mesothelioma samples were from normal tissue ("NT") and tumor tissue ("TT"), respectively. We used

anti-Dvl-3 antibodies ("Primary Ab mouse DVL3 1:400") to detect the Dvl-3 protein in these mesothelioma cells. The Western blot result shown on page 3 of **Exhibit 3** demonstrated overexpression of Dvl-3 protein in 3 out of 4 mesothelioma tumor cells (lanes marked "TT") compared to normal cells (lane marked "NT"). The result shown in **Exhibit 3** was obtained prior to December 2, 2002. Further, Figure 9 of the '037 Application, filed October 4, 2002 shows overexpression of Dvl-3 protein in cancer cells, specifically in several mesothelioma cell lines when compared to normal mesothelioma cells.

12. In addition, prior to December 2, 2002, we determined that some breast cancer cells also overexpressed Dvl-3 when compared to normal epithelial cells.

13. Prior to December 2, 2002 we had discussions as to how to inhibit expression of Dvl proteins, more specifically, a Dvl-3 protein. In particular we proposed inhibiting expression of the Dvl-3 protein using RNA interference (RNAi), more specifically using small interfering RNA (siRNA).

14. Prior to December 2, 2002, we designed and ordered small interfering RNAs (siRNAs) for inhibiting Dvl-3 expression in cancer cells overexpressing a Dvl-3 protein.

15. Prior to December 2, 2002, Kazutsugu Uematsu, who was a post-doctoral fellow working in our laboratory at this time under our supervision, ordered Dvl-3 siRNAs from Ambion, Inc. Austin, TX.

16. **Exhibit 4** shows the receipt of the Dvl-3 siRNAs ordered from Ambion, Inc., Austin, TX. All dates on **Exhibit 4** have been redacted. The order date for he Dvl-3 siRNAs was prior to December 2, 2002.

17. **Exhibit 4** shows (i) the target sequence name, "dv13," which should read "dvl3", (ii) the target sequence (5'-AACAAGATCACCTTCTCCGAG-3'), (iii) the sense sequence (5'-

CAAGAUCACCUUCUCCGAGtt-3' having "tt" added to its 3' end) and (iv) the antisense sequence of the target sequence (5'-CUCGGAGAAGGUGAUCUUGtt-3').

18. **Exhibit 5** shows a human Dvl-3 protein and nucleotide sequence obtained from GenBank accession No. NM_004423.

19. **Exhibit 6** shows the result of a sequence alignment of the target sequence (5'-AACAAGATCACCTTCTCCGAG-3') shown in **Exhibit 4** with the Dvl-3 nucleotide sequence of NM_00423 using BLASTN. This result demonstrates that the target sequence (5'-AACAAGATCACCTTCTCCGAG-3') and the corresponding antisense sequence of the target sequence (5'-CUCGGAGAAGGUGAUCUUGtt-3') as designed and ordered are found within the Dvl-3 nucleotide sequence.

20. **Exhibit 7** shows the result of a sequence alignment of the sense sequence (5'-CAAGAUCACCUUCUCCGAG-3'; missing the "tt" at the 3' end) with the Dvl-3 nucleotide sequence of NM_00423 using BLASTN. This result demonstrates that the sense sequence as designed and ordered is found within the Dvl-3 nucleotide sequence.

21. **Exhibits 1-7** are evidence of our conception of the invention, which was complete prior to December 2, 2002, the filing date of Alsobrook's '928 Application.

22. After conceiving of the invention, we worked diligently on the invention during the period of just prior to December 2, 2002, until our invention was actually reduced to practice and constructively reduced to practice with the filing of our '350 Application on July 31, 2003. **Exhibits 8-11** are provided as evidence of diligence and continuous activity relating to the invention. All dates on **Exhibits 8-11** have been redacted.

23. **Exhibit 8** is a page from Kazutsugu Uematsu's laboratory notebook. **Exhibit 8** shows a laboratory procedure to transfect Dvl-3 siRNA into cancer cells to inhibit the growth of the cancer cells, specifically lung cancer cell lines H460 and H1703 and osteosarcoma

cancer cell line Saos-2. We have shown that these cancer cells overexpress the Dvl-3 protein.

24. **Exhibit 9** is a print-out from our laboratory records showing that Dvl-3 siRNA inhibited the growth of the lung cancer cell line H1703, while a control siRNA had no significant effect.

25. **Exhibit 10** is a print-out from our laboratory records showing that Dvl-3 siRNA inhibited the growth of cancer cells, in particular the growth of lung cancer cells H460 and H1703 and the growth of the mesothelioma cell lines H513 and REN, while control siRNA had no such effect on these cancer cell lines.

26. **Exhibit 11** shows a Western blot analysis demonstrating that transfection of the Dvl-3 siRNA into the lung cancer cell line H1703 not only inhibited the growth of this cell line (see above), but also inhibited the expression of the Dvl-3 protein, while a control siRNA had no such effect.

27. We respectfully submit that the facts provided in this Rule 131 Declaration are sufficient to evidence that we completed conception of the invention as claimed prior to December 2, 2002, and that we worked diligently from just prior to December 2, 2002 until constructive reduction to practice on July 31, 2003.

Dated: 10-1-07

Biao HE

Dated: 10/1/07

Liang YOU

Dated: 9/30/07

Zhidong XU

Dated: 10/1/07

David M. JABLONS

61147062 v1

REDACTED

cDNA expression array REDACTED RNA

RNA samples meso. # 10799 111

#2 NP #2 MESO

3μl RNA for each sample

use column to purify the Labeled cDNA from unincorporated 32P nucleotides keep the probe at -20°C each probe volume is 70μl.

REDACTED

① add 130μl dmso to each probe tube
22μl 10x denaturing solution
68°C 20'

② warm up 10ml express Hyb + 100μl 10mg/ml salmon sperm DNA (105°C 5' first)

③ 9ko 2B, 4B membrane are placed in 80-90°C 0.5% SDS for 5'

REDACTED

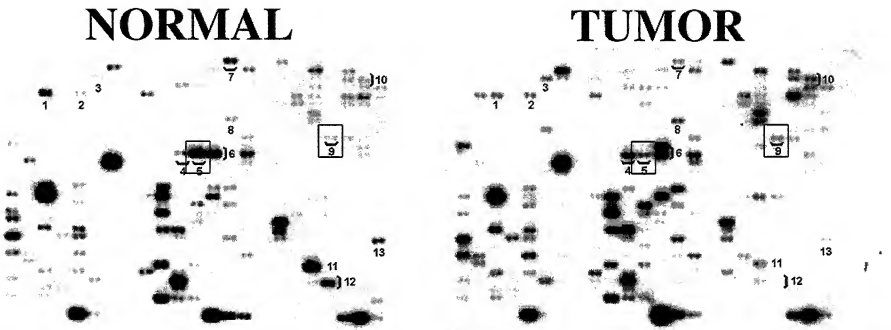
Wash membranes
Solution #1 20' 68°C x 4
" #2 20' 68°C x 1
2x SSC 5' room temp,

REDACTED

good result worked.

EXHIBIT 1

Identification of Dvl-3 Overexpression Using Microarray Gene Expression (Atlas Cancer Array)



cDNA Array Analysis. Representative cDNA spots (each shown in duplicate on the each membrane) that show differences are: 1, p21WAF/CIP1; 2, PCNA; 3, GRB2; 4, DAPK1; 5, SFRP2; 6, RhoA; 7, c-fos; 8, c-met; 9, DVL3; 10, IGFBP2; 11, EGR-1; 12, IL-6; 13, IRF-1.

REDACTED

Prepare protein extract from the Lung tissues samples.

1089 0280 TT

1089 0280 NT

1099 7021 Mouse

use 1 5ml protein extract buffer for each sample

REDACTED

Measure protein concentration

7021	Mouse	0.7	(5µl)	10 µg/µl
0280	TT	0.5	(10µl)	2.8 µg/µl
0280	NT	0.4	(10µl)	8 µg/µl

Run on 10% protein gel

ladder

ladder	9111 NP	9111 M	0280 NT	0280 TT	7021 M	9120 M	7020 N
(5µl)	21µl	5.1µl	6.25µl	13µl	7µl	10µl	21µl
5µg	15µg	15µg	5µg	5µg	30µg	15µg	15µg

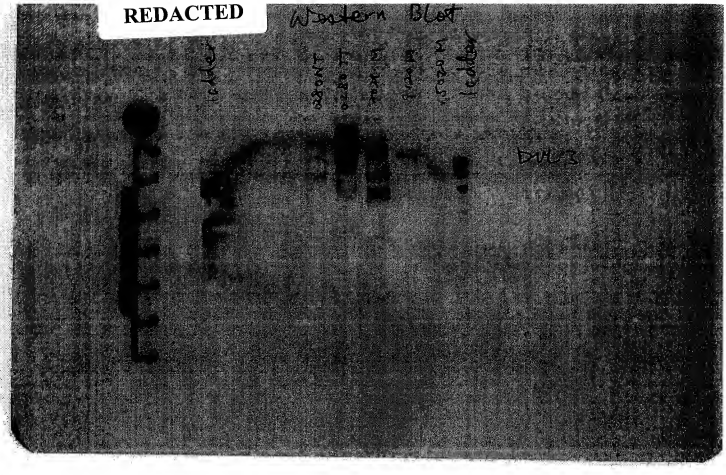
transfer o/n

REDACTED

primary Ab - mouse DVL 3 1:400

2nd Ab - mouse 1:2000

EXHIBIT 12


REDACTED
Western Blot
ladder
patient 1
patient 2
patient 3
patient 4
patient 5
ladder
DMD3

REDACTED

Gel 1 — from tissue

#	H2O	Protein 50µg	3x loading dipper
10899111 AT	7.5µl	5.5µl	7µl
10899111 TT	6.5µl	12.5µl	7
10899.021 NT	8.5µl	4.5µl	7
10899.021 TT	7.5µl	6.4µl	7
10899111 NT	2.4µl	6.6µl	7
10899111 TT	6.4µl	6.6µl	7
10899.20 NT	6.7	6.3	7
10899.20 TT	5.9	7.1	7

Gel 2 — cells

#	H2O	Protein 50µg	3x loading dipper
tissue 10895101 NT	8µl	5µl	7
cell 10899111	5µl	8µl	7
∨ MS-1	4.7	8.3	7
211 H	6.4	6.6	7
∨ H 2052	2.1	10.9	7
∨ 10899.020	3.4	7.6	7
∨ Met 5A	9.9	5.1	7
∨ H 593	5.2	7.8	7

EXHIBIT 3

REDACTED

Primary Ab ~~a~~- mouse DVL-3 1:400 O/N

REDACTED

Second Ab α mouse HRP labelled Ab 1:2000
5:20 pm - 6:20 pm

REDACTED

- Run a 10% protein gel 50μg protein for sample.

marker 1089911 → 1089102 1079911 1089120 108
NT, TT NT, TT NT, TT, NT, TT,

- Transfer 4°C, O/N, 40 volts.

REDACTED Wash w/ TBS-T, 2nd Ab
develop film

REDACTED

Prepare sample for frozen section (Tissues)

10795124 NL, LC	10895170 NL, LC
10897180 NL, LC.	10791270 NL, LC
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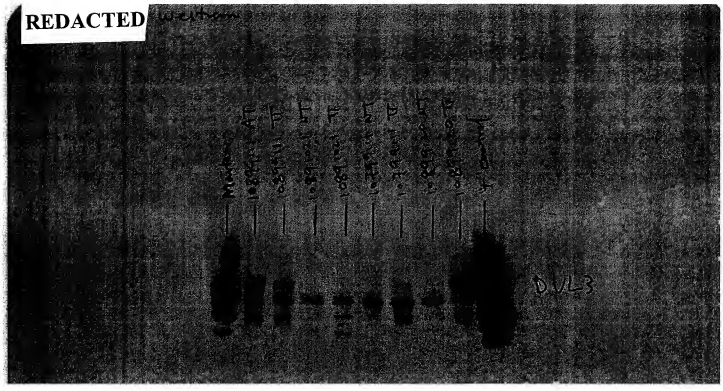
1089150 NL, LC	


REDACTED

Ambion®
THE RNA COMPANY®

Order ID
379825

Sequence ID*
671

CustomerName	PO Number	Institution	OrderDate
Kazutsugu Uematsu	1B017A2111RESEA	UCSF Cancer Center	REDACTED

See Sequence ID on back of box

Target Sequence Name
dv13

SiRNA Kit Lot #
072R03A

Target Sequence
5' AACAAGATCACCTTCTCCGAG 3'

Sense Sequence — **Seq. Lot #** 026178

5' CAAGAUCACCUUCUCCGAGtt 3'

Length	Molecular Weight	%GC	Ext. Coef.	1 OD(260)=
21	6589.2	48	198400 L/(mole-cm) *Using Nearest Neighbor equation	33.21 Micrograms / 5.04 nanomoles

OD260	Micrograms	Nanomoles
5.16	171.3636	26.0064

100 µM solution DW 260 µl

AntisenseSequence — **Seq Lot #** 026179

5' CUCGGAGAAGGUGAUCUUGtt 3'

Length	Molecular Weight	%GC	Ext. Coef.	1 OD(260)=
21	6726.2	48	207700 L/(mole-cm) *Using Nearest Neighbor equation	32.38 Micrograms / 4.81 nanomoles

OD260	Micrograms	Nanomoles
4.89	158.3382	23.5209

100 µM solution DW 235 µl

Scale	Purification
0.20 micromolar	PAGE

5' Modifications	Internal Modifications	3' Modifications
N/A	N/A	N/A

QC [signature] Date REDACTED

REDACTED

Ambion, Inc. • 2130 WOODWARD STREET • AUSTIN, TEXAS 78744-1832 • TEL: (512)651-0200 • TOLL-FREE: (800)888-8804 • FAX: (512)651-0201 • WWW.AMBION.COM

EXHIBIT 4

☐ 1: NM_004423. Reports Homo sapiens dish...[gi:41406096]

Order cDNA clone,
Links

Comment Features Sequence

```
LOCUS       NM_004423               5062 bp    mRNA    linear   PRI 27-JUN-2007
DEFINITION  Homo sapiens dishevelled, dsh homolog 3 (Drosophila) (DVL3), mRNA.
ACCESSION   NM_004423
VERSION     NM_004423.3  GI:41406096
KEYWORDS    .
SOURCE      Homo sapiens (human)
  ORGANISM  Homo sapiens
            Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi;
            Mammalia; Eutheria; Euarchontoglires; Primates; Haplorrhini;
            Catarrhini; Hominidae; Homo.
REFERENCE   1  (bases 1 to 5062)
  AUTHORS   Chan,D.W., Chan,C.Y., Yam,J.W., Ching,Y.P. and Ng,I.O.
  TITLE     Prickle-1 negatively regulates Wnt/beta-catenin pathway by
            promoting Dishevelled ubiquitination/degradation in liver cancer
  JOURNAL   Gastroenterology 131 (4), 1218-1227 (2006)
    PUBMED  17030191
  REMARK    GeneRIF: The facilitation of Prickle-1 on Dvl3 degradation and the
            suppression of beta-catenin activity and cell growth suggest that
            Prickle-1 is a negative regulator of the Wnt/beta-catenin signaling
            pathway
REFERENCE   2  (bases 1 to 5062)
  AUTHORS   Song,D.H., Sussman,D.J. and Seldin,D.C.
  TITLE     Endogenous protein kinase CK2 participates in Wnt signaling in
            mammary epithelial cells
  JOURNAL   J. Biol. Chem. 275 (31), 23790-23797 (2000)
    PUBMED  10806215
REFERENCE   3  (bases 1 to 5062)
  AUTHORS   Kishida,S., Yamamoto,H., Hino,S., Ikeda,S., Kishida,M. and
            Kikuchi,A.
  TITLE     DIX domains of Dvl and axin are necessary for protein interactions
            and their ability to regulate beta-catenin stability
  JOURNAL   Mol. Cell. Biol. 19 (6), 4414-4422 (1999)
    PUBMED  10330181
REFERENCE   4  (bases 1 to 5062)
  AUTHORS   Bui,T.D., Beier,D.R., Jonssen,M., Smith,K., Dorrington,S.M.,
            Kaklamanis,L., Kearney,L., Regan,R., Sussman,D.J. and Harris,A.L.
  TITLE     cDNA cloning of a human dishevelled DVL-3 gene, mapping to 3q27,
            and expression in human breast and colon carcinomas
  JOURNAL   Biochem. Biophys. Res. Commun. 239 (2), 510-516 (1997)
    PUBMED  9344861
REFERENCE   5  (bases 1 to 5062)
  AUTHORS   Semenov,M.V. and Snyder,M.
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TITLE Human dishevelled genes constitute a DHR-containing multigene family
JOURNAL Genomics 42 (2), 302-310 (1997)
PUBMED 9192851
REFERENCE 6 (bases 1 to 5062)
AUTHORS Pizzuti,A., Amati,F., Calabrese,G., Mari,A., Colosimo,A., Silani,V., Giardino,L., Ratti,A., Penso,D., Calza,L., Palka,G., Scarlato,G., Novelli,G. and Dallapiccola,B.
TITLE cDNA characterization and chromosomal mapping of two human homologues of the Drosophila dishevelled polarity gene
JOURNAL Hum. Mol. Genet. 5 (7), 953-958 (1996)
PUBMED 8817329
COMMENT REVIEWED REFSEQ: This record has been curated by NCBI staff. The reference sequence was derived from U75651.1 and D86963.1.
On Jan 30, 2004 this sequence version replaced gi:6806886.

Summary: This gene is a member of a multi-gene family which shares strong similarity with the Drosophila dishevelled gene, dsh. The Drosophila dishevelled gene encodes a cytoplasmic phosphoprotein that regulates cell proliferation.
COMPLETENESS: complete on the 3' end.

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	2832-3084	D86963.1	2832-3084	
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      421 ataacccatc ggagctgcca ccacctatgg agcgcacggg aggcatcggg gactcccgac
      481 ccccatcctt ccaccctcat gctggtgggg gcagccagga gaacctggac aatgacacag
      541 agacggactc tttggtgtct gcccagcgag agcggccacg ccggagggat ggcccagagc
      601 atgcaacccg gctaaatgga actgcgaagg gggaacggcg gcgagaacca gggggttatg
      661 atagctcatc cacccttatg agcagtgagc tggagaccac cagcttcttt gactcagatg
      721 aggatgactc caccagcagg ttcagcagct ccacagaaca gagcagtgcc tcacgcctga
      781 tgagaagaca caagcggcgg cggcggaagc agaaggtttc tcggattgag cggtcctcgt
      841 ccttcagcag catcacggac tccaccatgt cactcaacat catcacggtc actctcaaca
      901 tggaaaaata taacttcttg ggcatctcca ttgtgggcca aagcaacgag cgtggtgacg
      961 gcggcatcta cattggctct atcatgaagg gtggggccgt ggctgctgat ggacgcatcg
     1021 agccaggaga tatgttgtta caggtaaacg agatcaactt tgagaacatg agtaatgacg
```

```
1081 atgcagtccg ggtactgcgg gagattgtgc acaaaccggg gcccatcacc ctgactgtag
1141 ccaagtgctg ggacccaagt ccacgtggtt gcttcacatt gcccagggag gagcccatcc
1201 ggcccattga ccctgcggcc tgggtctccc acactgcagc catgaccggc accttccctg
1261 catacggcat gagccccctc ctgagcacca tcacctccac cagctcctcc atcaccagtt
1321 ccatccctga cacagagcgc ctagacgact tccacttgtc catccacagt gacatggctg
1381 ccatcgtaaa agccatggcc tcccctgaat cagggttgga ggtccgtgac cgcatgtggc
1441 tcaagattac catcctcaat gctttcatcg gctcagatgt ggtggactgg ctgtaccaca
1501 atgtggaagg cttcacggac cggagggagg cccgcaagta tgccagcaac ctgctgaaag
1561 ctggcttcat ccgccatacc gtcaacaaga tcaccttctc cgagcagtgc tactacatct
1621 tcggtgacct ctgcggcaac atggccaacc tgtctctcca cgatcacgat ggctccagtg
1681 gcgcctctga ccaggacaca ctggcccctt tgccgcaccc gggggccgcc ccttggccca
1741 tggctttccc gtaccagtac ccgccacccc cgcacccata caacccgcac ccgggcttcc
1801 cggagctggg ctacagctac ggcgggggca gcgccagcag tcagcacagc gaaggcagtc
1861 ggagcagtgg ctccaaccgt agcggcagcg atcggaggaa ggagaaggac ccgaaggccg
1921 gggactccaa gtccgggggc agcggcagcg aatcggacca caccacacgc agcagcctgc
1981 gggggccgcg ggagcgggcg cccagcgagc gctcagggcc ggcggccagc gagcacacct
2041 accgcagcca ccattccctg gccagcagcc ttcgcagcca ccacacacac ccgagctacg
2101 gtcctcccgg agtgccccct ctctacggcc ccccatgct gatgatgccc ccgccgccgg
2161 cggccatggg gccccagga gcccctccgg gccgcgacct ggcctcagtg cccccggaac
2221 tgaccgccag cagacagtcc ttccgcatgg ccatgggaaa ccccagtgag ttctttgtgg
2281 atgtgatgtg agcagggccc ctccccagc tccattccgc tcccacccca gccggctgcg
2341 ttcctctctc catccgtccg tctttttac tttgtctggt acctgaaagg gaaataaaag
2401 gaactaaatc caggtgcgct aactgctcgc agggtgctgc gagggtgggg tgcacctacc
2461 gattggctct gcagcccct aacctgcctc tggcccagt tcgtttcctc tgcccactaa
2521 tccctgcgca ggacttccca ggaccccttt tgtctctggg accagacttg ttggtgctac
2581 ccctactcc cctctgcaac ccccattttg ggagttgacc ccagcaatga ccttggtggc
2641 acgctcactc cctcattctc tcgtttcccc tttagctccc tttcaccatt tattcagcta
2701 catcatccct ctattaaccc caccccatca ggcacgtgtg caaacctctt gactttaccc
2761 cacattactg aaaccaaaat atatttgctt catctgcccc tactaaccat cccctgcct
2821 gctgcctcag tcctgcaacc taaagctgta gtcgcctcca atagccatcc atgccatccc
2881 tgcctgtgcc tagatcagag gcccagaggg ccccctcagt tgcctgagca gctggtggct
2941 tccaggggagc atctctgctc taccccctgcc ccatgcctgc cctgcgtgct ggttccttca
3001 gacccctaac cctactaacc agcaggctca tctcacctcc aggcctgaaa catttctttt
3061 ctttcttttt tcctccccca atttaccctg ggcctggagc agccaagaat ttcgggctgt
3121 ttgactttct gtgagccccc agcgagggga ggcccagcct cgcaggagac caggaacctt
3181 gcttcagcag cccctcaggg cttcccaagg atgtccagcc cccacaccca cacgttaaca
3241 taatgagtca ctaggcttct ggggagggcc caacttcacc catgcatgag agactctcct
3301 cctttccaga gagaatcgga tcgcaccacg tgtggcagcc tgcggcgggg ggaggggggc
3361 ctctttagct ctctttatct ttctctctca ctcatgtatg catacatgca cagagatgca
3421 tacacaggtg cctatgcaag ttcatttaag cctcagggct ggtcctcgcc caaagggctg
3481 gacctcccta atcctctcct aggttgtggg gctggtcccc tgacaccctt ctccccttcc
3541 tggtagacct taaacctcgc acacatgtcc ccagcatttt ctcacctgga taaagcccat
3601 aagctgggtc tcaggctggg ctcagcaaag gactcgcctt gcaaccgaca ggccattccc
3661 acccccacac acaacctccc ctgttttcac attcaccatg gcatccgaga gcaaggacac
3721 aggagcccac aggccagttg aggttgggca aggagacttc caggacttcc agacagagta
3781 ccaggttttа tttttcacct tattctctac tttaaacaaa tcataacttt ctctttaagc
3841 ctctgctata aattctcctg gctctcctgg gcttccatat tttgggggct ggggtgtcaa
3901 aagtgagatg aagttcttag ctccaggttt tggggtaaac caaggtagga acattttggc
3961 atttatttca attaacaata cttcctttgga cgggtgcggt ggctcacgtc tgtaatccca
4021 gcacttggg aggctgagac aggtggatca cttgaggtcg ggagttcgag accagcctgg
4081 ccaacatagc aaaacctgt ctctactaaa aatacaaaaa ttagatgggt gttttggcac
4141 ctgcctgtaa tctcagctac tcaggaggct gaggcacaag aatcgcttga atccaggaag
4201 caagcggagg ttgcagtgag ttgagatcgc actccagcct gggtgacaga gtgagattct
4261 gtctcaaaag caaactaaca aagaaaaaca atacttcctg gggttttggt gtgcagaggg
4321 ctttgttgga agtgtgactc aatcttgcct gccttctggg agctctagaa ttgttcccaa
4381 cccagtccat ggcttctagc caccactaca gggctgtttc atgtacttct ctctctgact
4441 ctgtcttgtc cgactctctt gagaatttct caacgattgc tcatgcctgt cagtatcagt
4501 gcttccatcg ttccatctttt gattcacttc tctttccttt ctatttactc ccaaaatgga
4561 gtcattcatc ctgatgtcct caattgctgc tgatatgctg gtgattccca aatacatagc
4621 tccaaccccc aacttccccc agactttaga tctgtattgg tattacctac tggacatctc
```

```
     4681 tatggacagt tccgtataga ctcaactcat ctgcccaacc aagtatgttc ctcctgaatt
     4741 cctctcctgg ttacttcatc acaatctaca taggctcacc agctagaaac atttatgagc
     4801 ttacattcct tcttcccata tcttatcagc atatcatatc catttcactc caacactctg
     4861 tcttgaattt ggccctccct ctcccctctc tactttaatt cattggagca tgggatttgg
     4921 agttaggtgg ttttgggttt gaattccagc tctactattt ttggttgtgt gatagagtta
     4981 tttaacctct ctgagcctca gttccctcgt atgtaaaatg atgataataa tacctacctc
     5041 acagggttgt tgtgaggatt ta
//
```

NCBI Blast 2 Sequences results

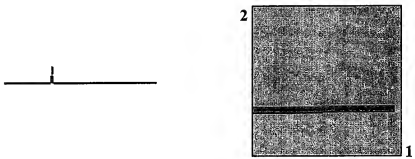
PubMed Entrez BLAST OMIM Taxonomy Structure

BLAST 2 SEQUENCES RESULTS VERSION BLASTN 2.2.17 [Aug-26-2007]

Match: 1 Mismatch: -2 gap open: 5 gap extension: 2
x_dropoff: 0 expect: 10.0000 wordsize: 11 Filter ☑ View option Standard
Masking character option X for protein, n for nucleotide Masking color option Black
☐ Show CDS translation Align

Sequence 1: lcl|1
Length = 21 (1 .. 21)

Sequence 2: lcl|65536
Length = 5062 (1 .. 5062)



NOTE:Bitscore and expect value are calculated based on the size of the nr database.

NOTE:If protein translation is reversed, please repeat the search with reverse strand of the query sequence.

```
 Score = 41.1 bits (21),  Expect = 0.019
 Identities = 21/21 (100%), Gaps = 0/21 (0%)
 Strand=Plus/Plus

Query  1     AACAAGATCACCTTCTCCGAG  21
             |||||||||||||||||||||
Sbjct  1584  AACAAGATCACCTTCTCCGAG  1604


CPU time:     0.09 user secs.       0.03 sys. secs          0.12 total secs.
```

EXHIBIT 6

Blast 2 Sequences results

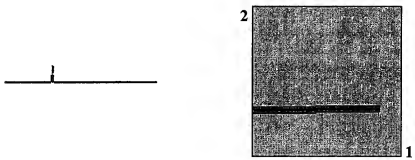
PubMed Entrez BLAST OMIM Taxonomy Structure

BLAST 2 SEQUENCES RESULTS VERSION BLASTN 2.2.17 [Aug-26-2007]

Match: 1 Mismatch: -2 gap open: 5 gap extension: 2
x_dropoff: 0 expect: 10.0000 wordsize: 11 Filter ☑ View option Standard
Masking character option X for protein, n for nucleotide Masking color option Black
☐ Show CDS translation Align

Sequence 1: lcl|1
Length = 21 (1 .. 21)

Sequence 2: lcl|65536
Length = 5062 (1 .. 5062)



NOTE:Bitscore and expect value are calculated based on the size of the nr database.

NOTE:If protein translation is reversed, please repeat the search with reverse strand of the query sequence.

```
 Score = 37.2 bits (19),  Expect = 0.27
 Identities = 19/19 (100%), Gaps = 0/19 (0%)
 Strand=Plus/Plus

Query  1     CAAGATCACCTTCTCCGAG  19
             ||||||||||||||||||||
Sbjct  1586  CAAGATCACCTTCTCCGAG  1604


CPU time:     0.10 user secs.       0.04 sys. secs          0.14 total secs.
```

REDACTED

※とりあえず ストリーク後に 280ml まで enhance した bacteria を 5ml とって
1500 rpm × 5 min いつもと違って LB が clear にならなかったが pellet が
できているので 上清を 5ml ピペットでとって 0.85ml を同分量で残す
150µl DMSO を入れて pellet を溶いて −80°Cへ (一番下の左から2番目 奥から
2番目の一番下の box)

siRNAのアニーリング

dvl3, 200µl	sense
200µl	anti-sense
200µl	5X buffer
400µl	DW
total 1000ml	⇒ これを 500µl ×2 とって

heat block を 使ってやるんと 500µl を 熱させたのでうまく
90°×1min
↓
1hr 37°C
(water bath を使って)
↓
これを −20°C に keep

dvl2 も 同様

上記 Oligofectamine で transfection するのに 24 well の cell 数 count

H460 $81 \times \frac{4}{9} = 36 \times 10^4 \times 0.2ml = 7.2 \times 10^4$ cells/well

H1703 $27 \times \frac{4}{9} = 12 \times 10^4 \times 0.2 ml = 2.4 \times 10^4$ 〃

ED , SD II. P14. P13

REDACTED transfection の ~~AA~~ Saos-2, 293T を harvest 41hr 後

RED. 顕鏡上は変化ないが 24 well の各 well を count

[illegible]

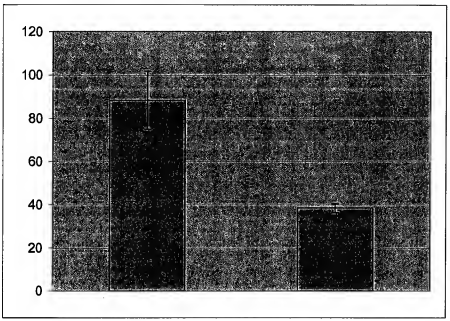
H1703 siRNA of Dvl ① $20 \times \frac{4}{9} \times 10^4 \times 0.5ml = 4.4 \times 10^4$ (stained 11)

REDACTED ② $27 \times \frac{4}{9} \times$ 〃 $= 6.0 \times 10^4$ (〃 8)

本日で 72hr

EXHIBIT 8

H1703	500/dish	
	controlsiRN	DvlsiRNA
colony cou	79	40
	98	37
ave	88.5	38.5
SD	13.43503	2.12132
TTEST	0.035065	



H1703/ **REDACTED**

hr	DvlsiRNA		control		DvlsiRNAa cont ave		DvlsiRNAd cont dev	
	A	B	A	B				
0	2.4	2.4	2.4	2.4	2.4	2.4	0	0
72	4.444444	6	18.66667	19.33333	5.222222	19	1.099944	0.471405
96	4.044444	8.222222	24.22222	24.44444	6.133333	24.33333	2.954135	0.157135
120	5.111111	9.333333	31.77778	35.77778	7.222222	33.77778	2.985562	2.828427
144	11.33333	11.55556	54.66667	65.55556	11.44444	60.11111	0.157135	7.699607

p
A549/ **REDACTED**

hr	DvlsiRNA		control		DvlsiRNAa cont ave		DvlsiRNAd cont dev	
	A	B	A	B				
0	0.4	0.4	0.4	0.4	0.4	0.4	0	0
50	1.35	2.24	1.83	1.59	1.795	1.71	0.629325	0.169706
74	5.555556	5.333333	3.555556	4	5.444444	3.777778	0.157135	0.31427
98	8.222222	8	10	11.11111	8.111111	10.55556	0.157135	0.785674
124	18	18.66667	14	12.22222	18.33333	13.11111	0.471405	1.257079
180	36.88889	36.22222	14	27.33333	36.55556	20.66667	0.471405	9.42809

H51301/ **REDACTED**

hr	DvlsiRNA		control		DvlsiRNAa cont ave		DvlsiRNAd cont dev	
	A	B	A	B				
0	3.555556	3.555556	3.555556	3.555556	3.555556	3.555556	0	0
48	3.777778	5.111111	3.777778	4.222222	4.444444	4	0.942809	0.31427
109	6	2.444444	4.222222	3.555556	4.222222	3.888889	2.514157	0.471405
144	3.777778	3.111111	7.555556	4.666667	3.444444	6.111111	0.471405	2.042753
192	5.555556	5.555556	9.555556	10.66667	5.555556	10.11111	0	0.785674
216	5.333333	7.333333	13.77778	17.11111	6.333333	15.44444	1.414214	2.357023

REN **REDACTED**

hr	DvlsiRNA		control		DvlsiRNAa cont ave		DvlsiRNAd cont dev	
	A	B	A	B				
0	2.666667	2.666667	2.666667	2.666667	2.666667	2.666667	0	0
48	9.111111	10.44444	11.11111	8	9.777778	9.555556	0.942809	2.199888
72	20.88889	13.77778	16	17.77778	17.33333	16.88889	5.028315	1.257079
96	18.66667	18.22222	27.55556	26.88889	18.44444	27.22222	0.31427	0.471405
120	24.66667	27.11111	29.33333	33.77778	25.88889	31.55556	1.728483	3.142697

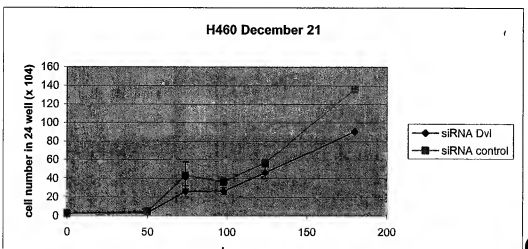
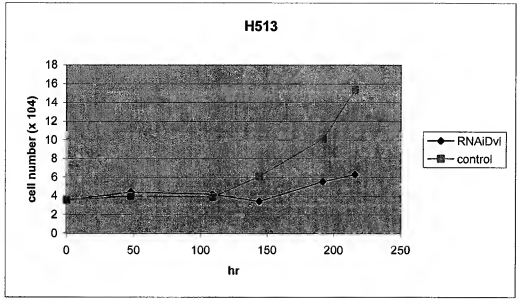
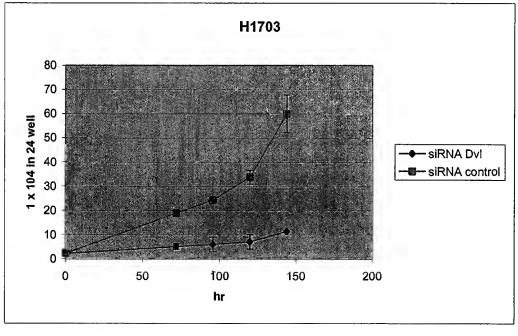


EXHIBIT 10

hr


H513
cell number (x 104)
18
16
14
12
10
8
6
4
2
0
0
50
100
150
200
250
hr
RNAiDvl
control


H1703
1 x 104 in 24 well
80
70
60
50
40
30
20
10
0
0
50
100
150
200
hr
siRNA Dvl
siRNA control

REDACTED

p H460

	hr	DvlsiRNA A	DvlsiRNA B	control A	control B	DvlsiRNAa cont ave		DvlsiRNAd
	0	7.2	7.2	7.2	7.2	7.2	7.2	0
0.003751	72	45	43	47	70.5	44	58.75	1.414214
0.012954	96	51.5	51	66	70	51.25	68	0.353553
0.011781	120	79	72.5	88.5	90	75.75	89.25	4.596194
0.01229	144	110	108	133.5	143	109	138.25	1.414214

REDACTED

H460/

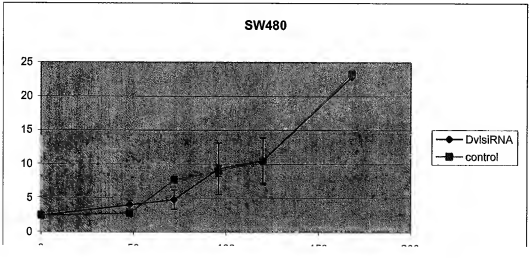
	hr	DvlsiRNA A	DvlsiRNA B	control A	control B	DvlsiRNAa cont ave		DvlsiRNAd
	0	2.3	2.3	2.3	2.3	2.3	2.3	0
0.870688	50	3.12	3.12	3.87	5.12	3.12	4.495	0
0.021508	74	30.5	22	53.5	33	26.25	43.25	6.010408
0.049745	98	24	29	36	37.5	26.5	36.75	3.535534
0.031494	124	51	42	54.5	59.5	46.5	57	6.363961
0.140284	180	90.5	90.5	136.5	136.5	90.5	136.5	0

REDACTED

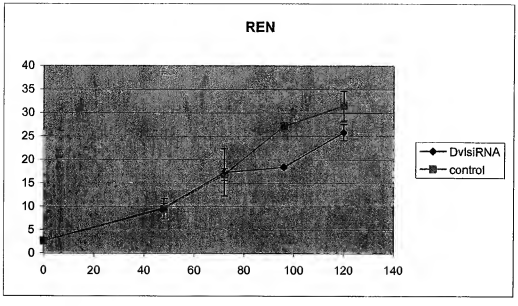
SW480

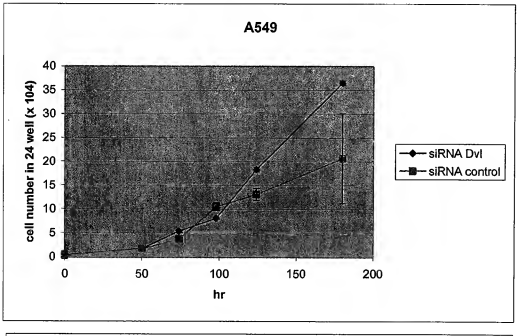
	hr	DvlsiRNA A	DvlsiRNA B	control A	control B	DvlsiRNAa cont ave		DvlsiRNAd
	0	2.4	2.4	2.4	2.4	2.4	2.4	0
0.591752	48	3.555556	4.444444	2.444444	3.111111	4	2.777778	0.628539
0.870781	72	5.777778	3.777778	7.333333	8	4.777778	7.666667	1.414214
0.213854	96	6.666667	12	9.555556	8.444444	9.333333	9	3.771236
0.014548	120	12.88889	8.222222	12.88889	8	10.55556	10.44444	3.299832
0.04262	168	22.88889	22.88889	23.33333	23.33333	22.88889	23.33333	0

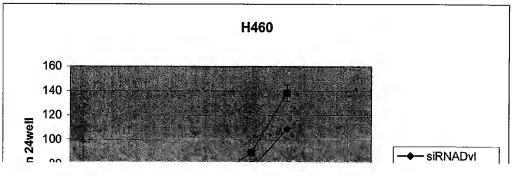
0.90755
0.914564
0.002077
0.155031

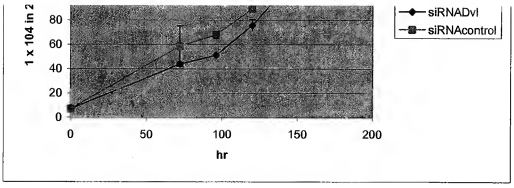



0
50
100
150
200


REN
40
35
30
25
20
15
10
5
0
0
20
40
60
80
100
120
140
DvlsiRNA
control


A549
cell number in 24 well (x 104)
40
35
30
25
20
15
10
5
0
0
50
100
150
200
hr
siRNA Dvl
siRNA control


H460
160
140
120
100
siRNADvl


1 x 104 in 2
80
60
40
20
0
0
50
100
150
200
hr
siRNADvl
siRNAcontrol

cont dev

0
16.61701
2.828427
1.06066
6.717514

cont dev

0
0.883883
14.49569
1.06066
3.535534
0

cont dev

0
0.471405
0.471405
0.785674
3.456966
0

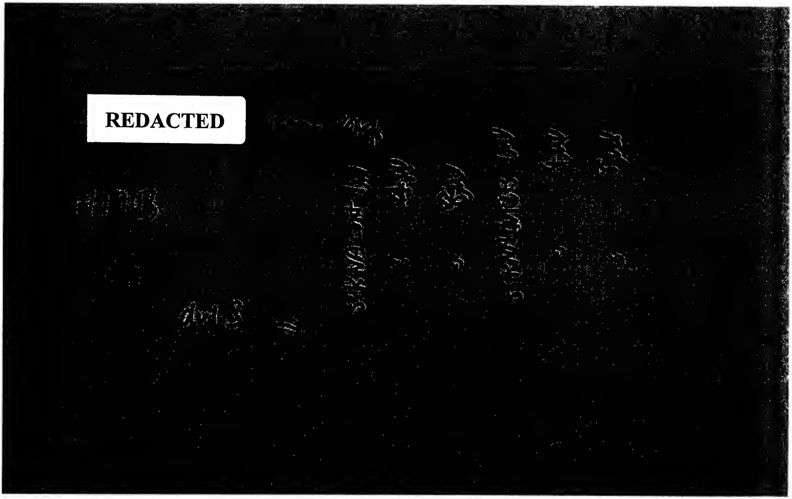


EXHIBIT
11